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THE LIGHT-DARK CYCLE AND A NONLINEAR ANALYSIS OF LUNAR PERTURBATIONS AND BAROMETRIC PRESSURE ASSOCIATED WITH THE ANNUAL LOCOMOTOR ACTIVITY OF THE FROG, *RANA PIPIENS*

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During the course of study on intestinal calcium transport in the frog *Rana pipiens,* it became apparent that maximal transport activity in April to June occurred during the nocturnal hours (Robertson, 1976). This period of increased physiological activity of the digestive system may be related to feeding behavior, but at present few studies have been made to determine the activity patterns of the leopard frog. Observations of frogs as a group in temperate zones show that activity is present during the day as well as at night, depending upon the species (Wright and Wright, 1949). At lower latitudes, as in Panama, *Bufo marinus* is active primarily during the day (Park, Barden and Williams, 1940); however, Jaeger, Haitman and Jaeger (1976) noted in the Panamanian frog, *Colostethus,* a bimodal activity pattern with maximums at 0830 and 1630 hr. This activity pattern was coincident with the crepuscular activity of the primary food source, dipterans and coleopterans.

With respect to the Ranidae group, *Rana temporaria* displays higher locomotor activity at night (Chugunov and Kuznetsov, 1972), while *Rana esculenta* is active throughout the day (Kuznetsov, Chugunov and Brodskii, 1972). Dole (1972) noted that newly metamorphosed specimens of *Rana pipiens* were more active after nocturnal rains. The food gathering activity patterns of adult *Rana pipiens* may be inferred from the stomach contents which contain those insects most commonly available at various times of the feeding season (April to October) (Whitaker, 1961; Linzey, 1967). Diet appears to be a reflection of the availability of various insects rather than preference and includes species of Coleoptera (beetles, weevils), Hymenoptera (ants), and Homoptera (aphids, leafhoppers). Of these, beetles (Oldryd, 1960) and ants (Skaife, 1961) display increased activity at night.

When food is made available at a preset period of time, the bullfrog, *Rana catesbeiana,* exhibits increased activity presumably in anticipation of food availability, suggestive of a "conditioned" response (van Bergeijk, 1967).

In spontaneous or nonconditioned activity, amphibians appear to be sensitive not only to the general environment, such as season and rainfall (Gibbons and Bennett, 1974), but to solar and lunar cues for orientation and migration (Ferguson, Landreth and Turinipseed, 1965; Ferguson and Landreth, 1966; Landreth and Ferguson, 1966, 1967; FitzGerald and Bider, 1974). When maintained in a closed environment away from visual cues, the activity of salamanders (*Triturus*) appears to be modified by a lunar influence (Ralph, 1957), which may also influence physiological activity, such as oxygen consumption (Brown, Webb, Bennett and Sanders, 1955). In an attempt to provide an overview of the diurnal activity of physiological processes of the digestive system, a year-long study was conducted on the spontaneous activity patterns of adult male *Rana pipiens* exposed to the change in outdoor ambient lighting conditions.

MATERIAL AND METHODS

Adult male specimens of *Rana pipiens* (Northern variety) of 40–60 g body weight were obtained commercially (Bay Biological, Canada) throughout the period of study from March, 1976, to February, 1977, with studies conducted in Syracuse, New York (Lat. 43° 03′ N). Frogs were unfed and maintained in a continuous change of fresh water (20–24° C) and exposed to outdoor ambient diurnal lighting conditions for one week prior to use. After this period, groups of 8–10 frogs were placed in the detection apparatus described below in a relatively quiet room. The frogs were exposed to southern ambient lighting (300–400 lux max). The surrounding yearly temperature was maintained at 19.3 ± 3.1° C and only significantly elevated above the yearly mean in September. Newly acclimated frogs were introduced into the apparatus at irregular intervals after 10–20 days. Since Brown, Webb and Macey (1957) had noted that barometric pressure may affect biological activity in amphibians, local barometric pressure at 1200 hr was recorded on a recording aneroid barometer throughout the course of study.

Detection apparatus

The spontaneous locomotor activity of groups of frogs was monitored by the detection of vertical water movement in an isolated translucent plastic chamber (33 × 23 × 10 cm). The apparatus consisted of a plastic float connected to a pivoted transverse rod through which a vertical water displacement of 2.0 ± 0.25 mm was multiplied by a factor of five. A contact switch at the end of the rod completed a circuit with an event marked on a strip recorder. The switch interval was adjusted daily to maintain a constant sensitivity.

Analysis of data

Locomotor activity (LA) was recorded as the number of events/hour and the number of events/day. Hourly variations were reduced by averaging the data for two hour periods. Further, the relative activity (per cent activity/two hour period) was calculated from the total number of events/day. Since lunar perturbations appeared to influence amphibian behavior (Ralph, 1957), identification of coherent patterns of locomotor activity was facilitated by the construction of isograms of the relative levels of activity within a lunar month. The mean relative activity pattern for a "typical lunar month" was calculated by deriving the mean relative activity (per cent/two hours) of corresponding lunar days over a three to four lunar month period. Lines were connected between equal levels of activity (5% intervals) to provide a "contour map" which would emphasize basic activity patterns.

The presence of a lunar influence during the diurnal and nocturnal periods was determined by a procedure described by Brown *et al.* (1955). High transit

(HT; time of moon at zenith) advances 50 minutes each day, and completes a cycle in about 29.53 days (synodical lunar month). The absolute levels of locomotor activity at HT for each day were then tabulated into a single column (Column 3) with the data of the subsequent time period shifted accordingly to the right. This procedure enhances any lunar effect associated with the time of HT that is superimposed upon the general level of activity. The times of transit were obtained from the *American Ephemeris and Nautical Almanac* (1976, 1977, pp. 52–67), and all time periods designated in Eastern Standard Time. Day of lunar month is designated as (NM + day).

Period analysis

A computer program was designed to identify and analyze the times of maximal activity with respect to a specific reference date. Since most activity was during the nocturnal period, the time of maximal locomotor activity (LA_{max}) was recorded as the time (hours) before (−) or after (+) 0000 hr. For example, a LA_{max} period of activity at 2000 hr was recorded as −4 (hr), while an activity peak at 0400 hr was recorded as +4 (hr). Initial observations suggested that the time of LA_{max} occurred at a different time each day but was repeated several days later at a similar time period. This could best be visualized as an oscillatory cycle with the function: $LA_{max} = M + \cos(\omega t - \phi)$, where the cyclic parameters are M (mean of all LA_{max} time values), A (amplitude of the oscillation), and ϕ (arcophase or phase angle, which is the day in the cycle in which LA_{max} occurs at the maximal positive hour after 0000 hr), after a specific reference date expressed in degrees. The reference date was taken as day 1 (NM + 1) of a lunar month on 4/1/76. The angular frequency $\omega = 360°/t$, where t = number of days in the cycle. For cyclic

TABLE 1

Monthly locomotor activity levels of adult male frogs, Rana pipiens.

Month	N (Days)	Events/day ± s.e.m.	Temp. range ° C (min-max)	Barometric pressure (mm Hg)*
January	17	539 ± 78	15.0–18.0	756.4 ± 7.2
February	28	420 ± 60	15.5–18.0	759.5 ± 7.3
March	11	297 ± 14	15.0–17.0	761.2 ± 6.0
April	28	373 ± 26	16.0–18.5	762.3 ± 3.3
May	30	387 ± 52	18.0–20.0	760.0 ± 5.8
June	21	405 ± 50	20.0–23.0	762.8 ± 4.2
July	10	286 ± 30	22.0–23.0	760.2 ± 4.4
August	29	281 ± 34	22.0–23.0	763.3 ± 4.0
September	29	211 ± 36	23.0–26.0	762.5 ± 5.2
October	31	256 ± 29	20.0–22.0	762.0 ± 6.8
November	30	146 ± 31	17.0–20.0	760.5 ± 5.7
December	14	159 ± 45	15.0–18.0	759.7 ± 8.7
Total annual mean	278	313 ± 15	19.4 ± 3.1	761.0 ± 6.1

* Mean pressure at 1200 hr.

analysis of LA_{max}, the time of maximal activity was paired with the number of the day after the reference date. The data was then tested with the cosine function above with hypothetical wave functions of various periods where $t = 5$ to 90 days for the monthly analysis and cycles up to 360 days for all data collected for the entire year. The procedure was conveniently done through a linear transformation of the cosine time parameter in which the data was analyzed as a simple linear regression. The "best fit of the data" was expressed as the maximum positive value of the correlation coefficient (r) at a specific ϕ. This value at ϕ was obtained by calculating the values of r at 10° increments through the entire wave function of 360° for each hypothetical cycle. The resulting maximum values of r for the range of cycles represents a spectrum in which the t statistic was calculated and $P \leq 0.01$ was considered a significant cycle. Possible correlations of barometric pressure and the daily absolute levels of locomotor activity on the cyclic spectrum were conveniently analyzed by processing the data only for those days when the values were above or below the annual mean. All data expressed in the text are mean ± s.d. unless otherwise stated, and $P \leq 0.01$, determined from a simple t-statistic between values, was considered significant.

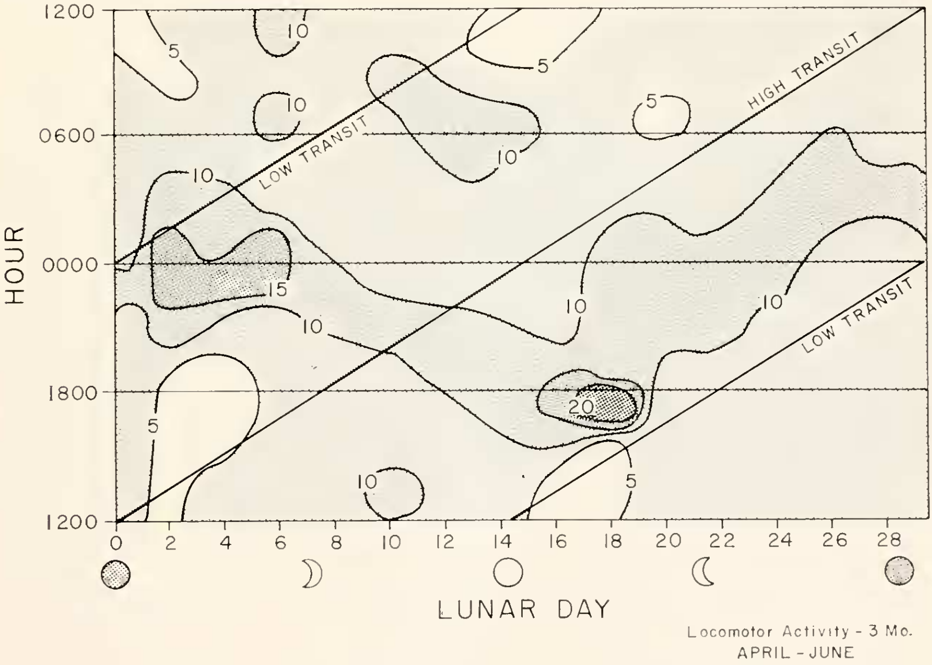


FIGURE 1. Isogram of relative locomotor activity (% activity/two hours) during April to June based upon the mean activity levels of corresponding lunar days over a three lunar month period. A coherent sinusoidal pattern is apparent which oscillates within the lunar month to reflect high activity at 0000 hr near New Moon which shifts to 1800 hr at a time of Full Moon. Additional activity at 0600 hr at Full Moon indicates a bimodal diel pattern. Diagonal lines represent times of high and low lunar transit.

RESULTS

General activity patterns

Since 8–10 frogs were continuously monitored, the locomotor activity (LA) in this study is group activity in contrast to activity of individual frogs. Such activity throughout the year (total of 278 days) revealed changes in the relative levels of hourly activity from month to month which did not appear to be related to the absolute monthly activity level or the ambient temperature. The yearly mean temperature was maintained at 19.4 ± 3.1° C and only significantly elevated above the mean in September (Table I). The mean daily activity levels in the calendar months of November, December and January showed significant deviations from the mean daily activity levels of 313 ± 115 events/day for the entire year. No significant correlation existed between the mean daily activity for each month and the corresponding mean monthly ambient temperature ($r = -0.395$) or the prevailing mean monthly barometric pressure ($r = -0.421$).

Chi square analysis of the relative hourly activity for each month showed no significant variation from "random" activity (8.3% activity/two hours) in the months from October through March, while a significant nonrandom light-dark response pattern of activity was apparent from April through September. Further analysis of this nonrandom period based upon monthly relative activity isograms

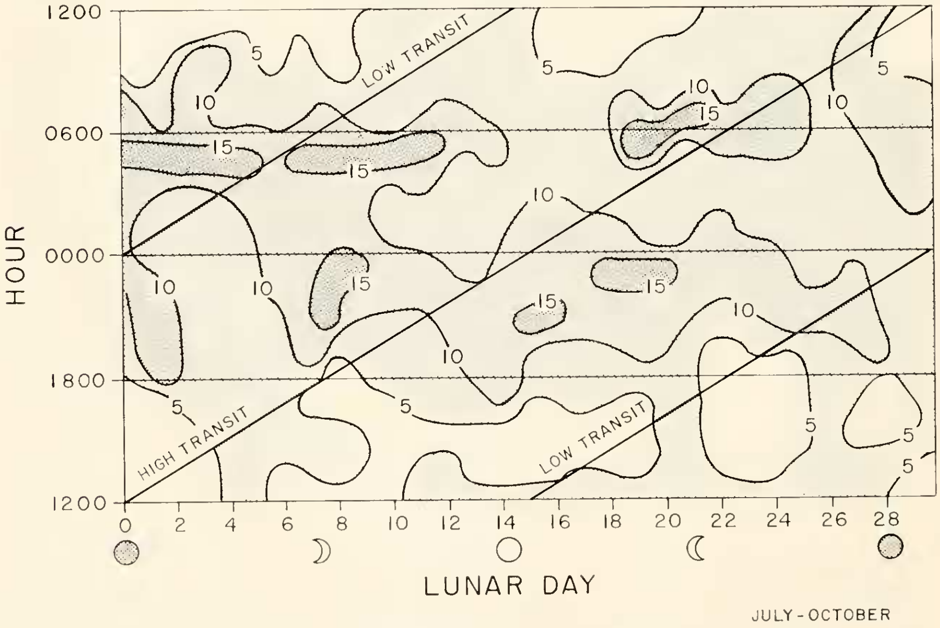


FIGURE 2. Isogram of relative locomotor activity (% activity/two hours) for a three lunar month period from July to October. Elevated activity (> 10%) is primarily nocturnal while > 15% activity is observed between 2100–0000 hr and 0400–0600 hr which reflects a bimodal diel activity pattern. Diagonal lines indicate time of high and low lunar transit.

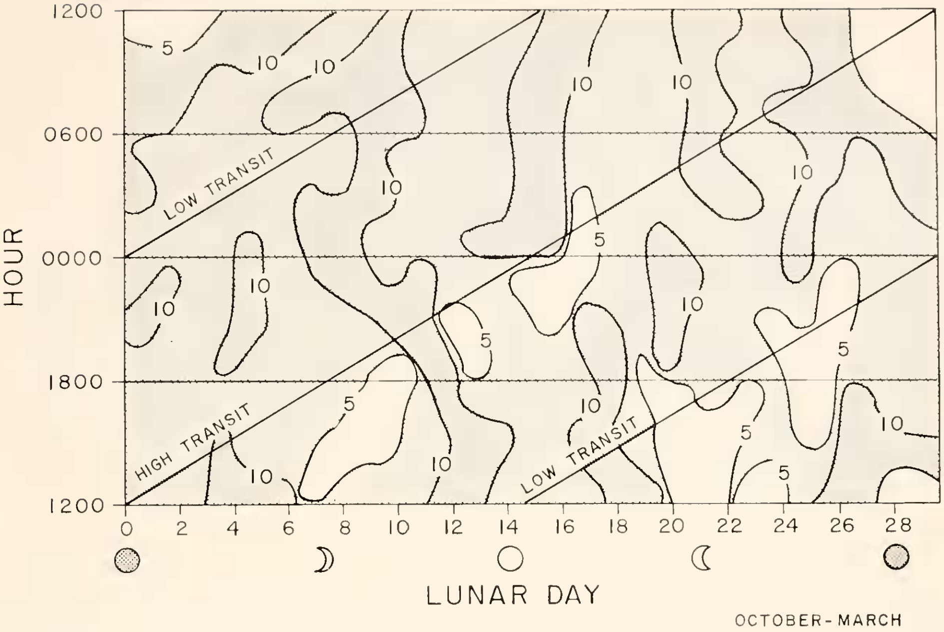


FIGURE 3. Isogram of relative locomotor activity (% activity/two hours) from October to March based on data of four lunar months. Activity is randomly distributed throughout the 24 hr period each day with no significant diurnal-nocturnal pattern evident. Diagonal lines indicate times of high and low lunar transit.

revealed a change in pattern during July. Thus, three major periods were identified, the first nonrandom period from April through June (79 days), a second nonrandom period from July to the first New Moon in October (90 days), and the third "random" period from New Moon in October through March of the following year (109 days).

The composite "lunar month" isogram constructed for the April to June period (three lunar months) revealed a coherent sinusoidal pattern with periods of increased LA (> 10%/two hours) near 0000 hr at New Moon (Fig. 1). This pattern shifted toward early evening hours during the progression of the lunar month to exhibit maximal LA at 1800 hr at Full Moon (NM + 15). In addition, a second period of increased activity was observed at 0600 hr which indicated a bimodal diurnal pattern. During the remainder of the month, the primary activity pattern returned toward 0000 hr and continued the shift to 0300 hr at the end of the lunar month. Thus, a single mode of activity appeared from NM + 16 to NM + 5 of the following lunar month and a bimodal activity pattern from NM + 6 to NM + 15.

The isogram based upon a composite of three lunar months of the second period (July through October) revealed a prominent bimodal diel pattern which was maximal at 0400–0600 hr with a minor maximum at about 2200 hr (Fig. 2). The major early morning maximum coincides with the time of sunrise (0430–0600 hr)

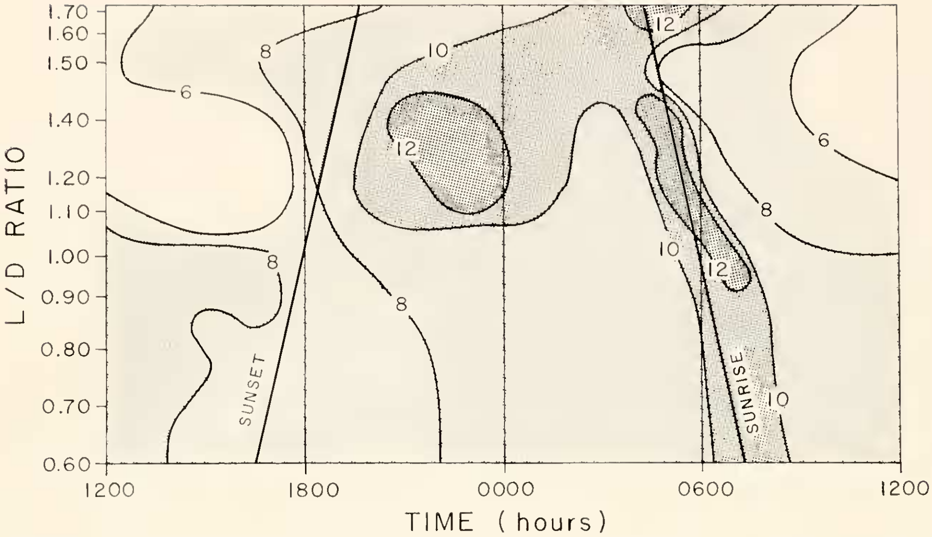


FIGURE 4. Isogram of relative locomotor activity (%/two hours) in adult male *Rana pipiens* as a function of the prevailing L:D ratio over the course of an entire year. During winter with an L:D ratio < 1.0, activity is not defined into a significant diurnal-nocturnal pattern although a nonsignificant increase is observed at sunrise. An L:D ratio between 1.0–1.45 is coincident with a significant increase in nocturnal activity between 2100–0000 hr and during the hours at sunrise. Above an L:D ratio of 1.5, frogs are most active at sunrise with little activity (< 6%) during daylight hours.

during this time of year. The sinusoidal pattern of the previous period did not appear to be dominant.

The third period (October through March), classified as random activity, was substantiated by the composite isogram based upon four lunar months (Fig. 3). No coherent daily pattern was apparent and the overall pattern was characterized by higher levels (> 10%/two hours) of activity present during the daylight hours, and low activity levels (< 5%/two hours) at night.

The change in pattern appeared to be related to the seasonal alterations in the L:D ratio as depicted in Figure 4. An isogram based upon the relative mean LA/two hours for each calendar month as a function of the prevailing L:D ratio revealed that random LA from mid-October to March occurred when the L:D ratio was < 1.0. As the L:D ratio increased to 1.0, increased LA was more apparent at time of sunrise and became maximal between 2100 and 0000 hr up to the time when the L:D ratio was < 1.45. Above this ratio the LA was predominately in the hours near sunrise with < 6% of the activity occurring during the mid-daylight hours.

Lunar influence

The relationship of lunar position on hourly LA was analyzed by columnating the absolute level of activity for each two hour segment during the time of High

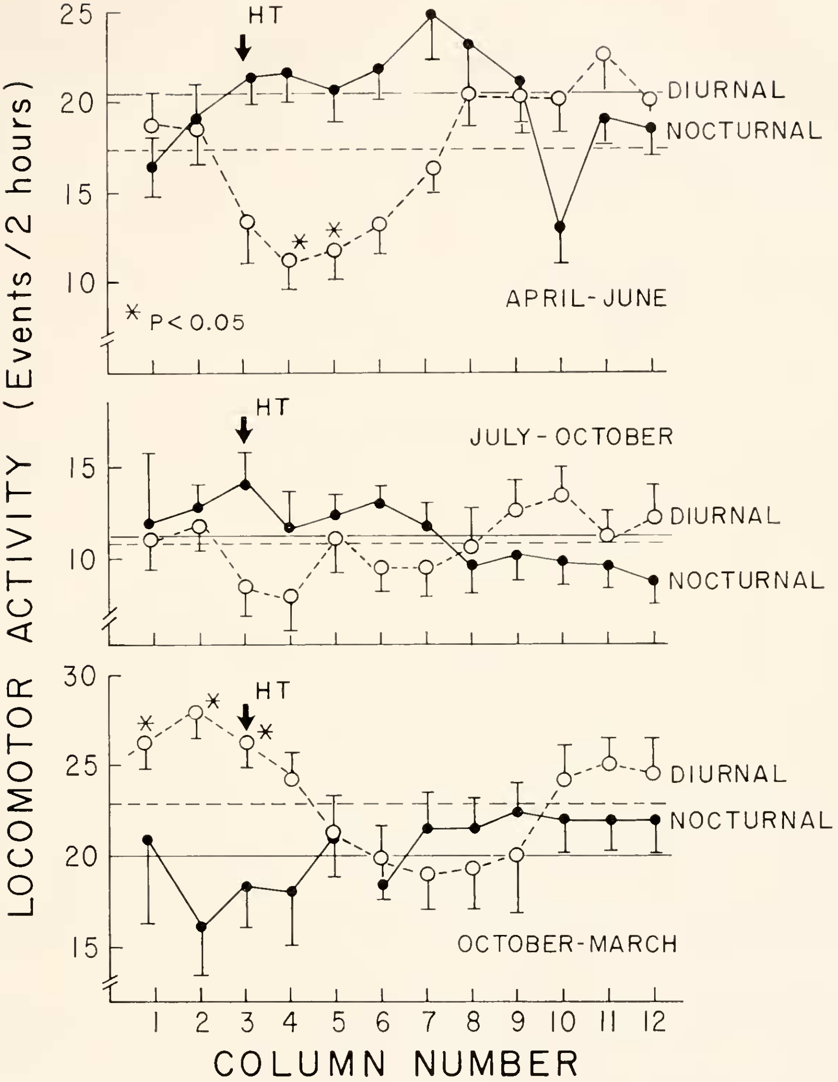


FIGURE 5. Relationship of high lunar transit (HT in column 3) on absolute levels of activity (number of events/two hours) during daylight (open circles with dashed line) and nocturnal (solid circles with solid line) hours during three major periods of the year. In April to June, HT during nocturnal period had no significant influence on activity level from nocturnal mean (solid line), while six hours after HT during diurnal period, activity was significantly decreased when compared to daily mean (dashed line). In July to October mean diurnal (dashed line) and nocturnal (solid line) activities were significantly lower than activity in preceding and subsequent monthly periods, and high transit was not associated with any significant change in mean hourly activity. In October to March mean nocturnal activity (solid line) was significantly below mean diurnal activity (dashed line) and activity four to six hours prior to HT during daylight was significantly above diurnal mean. Each point is mean ± s.e.m.

Transit (HT) (Column 3) when transit occurred either during the diurnal or nocturnal period (Fig. 5). During April, for example, HT advanced through the nocturnal period in 14 days, and through daylight in 16 days. For the entire April to June segment the time of HT was not coincident with any significant change in LA at any time during the dark from the mean nocturnal level of 20.4 ± 3.3 events/two hours. When transit occurred during daylight hours there was a significant decrease for six hours after HT below the diurnal mean of 17.3 ± 3.7 events/two hours. Further, the mean daylight level of activity was significantly below the nocturnal activity level.

High transit in July to October was not coincident with any hourly differential effect on diurnal or nocturnal activity levels, nor were the mean activity levels different from one another [(11.3 ± 1.9 (nocturnal) *vs.* 10.9 ± 1.7 events/two hours (diurnal)]. During this period, the total number of events/day (254 ± 17 s.e.m.) was significantly below the yearly mean of 313 ± 15 s.e.m. events/day.

The association of HT with decreased activity observed in April-June was reversed during the period from October to March. When HT occurred during daylight there was a significant increase in LA six hours prior to transit above the mean level of 22.9 ± 3.9 events/two hours which was also elevated above the nocturnal mean of 20.2 ± 2.2 events/two hours.

Period analysis

The characteristics of the FORTRAN Program employed in this study are such that insignificant values of r will be generated if data cannot be fitted to any sine wave by least squares or if LA_{max} is a linear function (*e.g.*, LA_{max} occurs at the same time each day). When LA_{max} was analyzed across a spectrum of hypothetical wave lengths at five day intervals, the presence of significant ($P \leq 0.01$) oscillatory patterns appeared as peaks of increased values of r. This procedure performed on each of the three monthly segments and for the entire year revealed the presence of cycles indicative of coherent and organized behavior patterns. Further processing of data based upon the relative levels of barometric pressure and locomotor activity (events/day) above or below the annual mean (Table I) altered the values of r (and degree of significance) of specific cycles. Under these circumstances the phase angle (ϕ) was identical to each base cycle for the recalculation of r.

In April to June (Fig. 6) the base spectrum for all data indicated the presence of a 28 day and 50 day cycle. The 28 day cycle where $\phi = 0$, began each cycle on the first day of New Moon during this period, and became a more significant cycle when analyzed for those days of low barometric pressure (LBP); whereas the 50 day cycle which began 14 days into the lunar month was more prominent under conditions of high barometric pressure (HBP). On days of low locomotor activity (LLA) a slight shift in the cycle occurred with the increase in the correlation coefficient to $r = 0.641$ with other peaks at 15 and 60 days.

In July to October, two additional shorter cycles were noted in addition to those seen in April to June (Fig. 7). The 5 day cycle was more significant under conditions of LBP, while the 15 day cycle was more prominent when tested on days of HBP. As noted in the April to June spectrum, the 30 and 45 day cycles

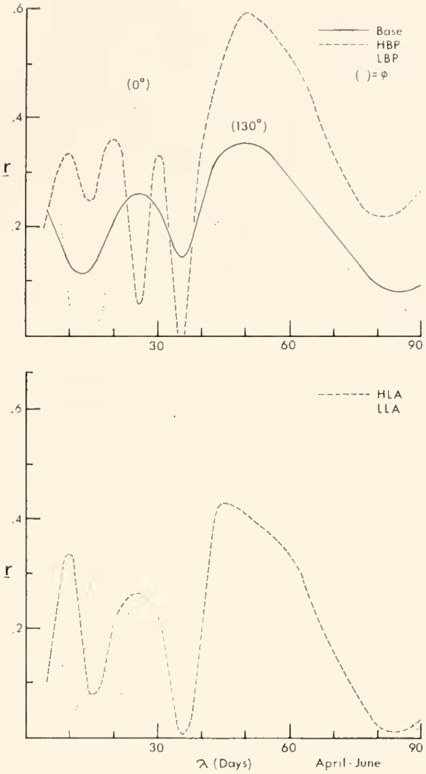


FIGURE 6. Upper graph shows a period analysis of times of LA_{max} during April to June depicted by the solid line indicating two significant cycles at 28 and 50 days. The resulting spectrum after analysis of data on days of low barometric pressure (LBP) at identical ϕ shows enhancement of the 28 day cycle, while on days of high barometric pressure (HBP) the 50 day cycle was the most significant. Lower graph shows a similar analysis on days in which activity was lower than the series mean (LLA) a 30 day cycle was most significant, whereas high locomotor activity (HLA) was associated with the 50 day cycle.

were more prominent under conditions of HBP. Examination of HLA revealed a significant enhancement of the 15, 25, and 50 day cycles, while the 5 day cycle was dominant on days of LLA.

For the period from October to March (Fig. 8) the base spectrum displayed cycles at 15, 30, and 50 days in which only the 50 day cycle showed a significant enhancement under conditions of lower than average barometric pressure. Higher than average locomotor activity was coincident with the 15 and 65 day cycles while LLA was associated only with the 30 day cycle.

When all data for the entire year was examined in the same manner (Fig. 9), only the significant cycles whose phase angle was relatively constant throughout the year could be detected. The significant base cycles present were those at 30, 55, 105, and 160 days. On days of HBP the 105 and 160 day cycles were most

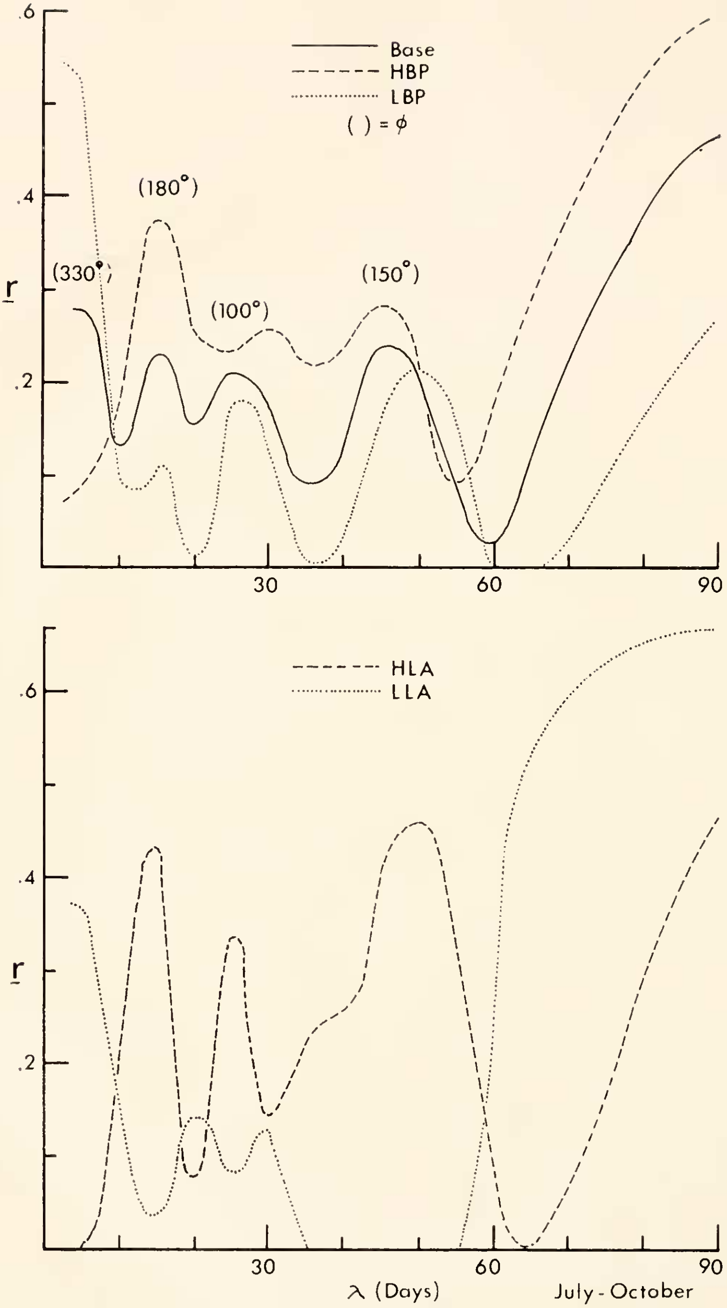

Base
HBP
LBP
( ) = φ
(180°)
(330°)
(100°)
(150°)
r
.6
.4
.2
30
60
90
HLA
LLA
r
.6
.4
.2
30
60
90
λ (Days)
July - October

TABLE II

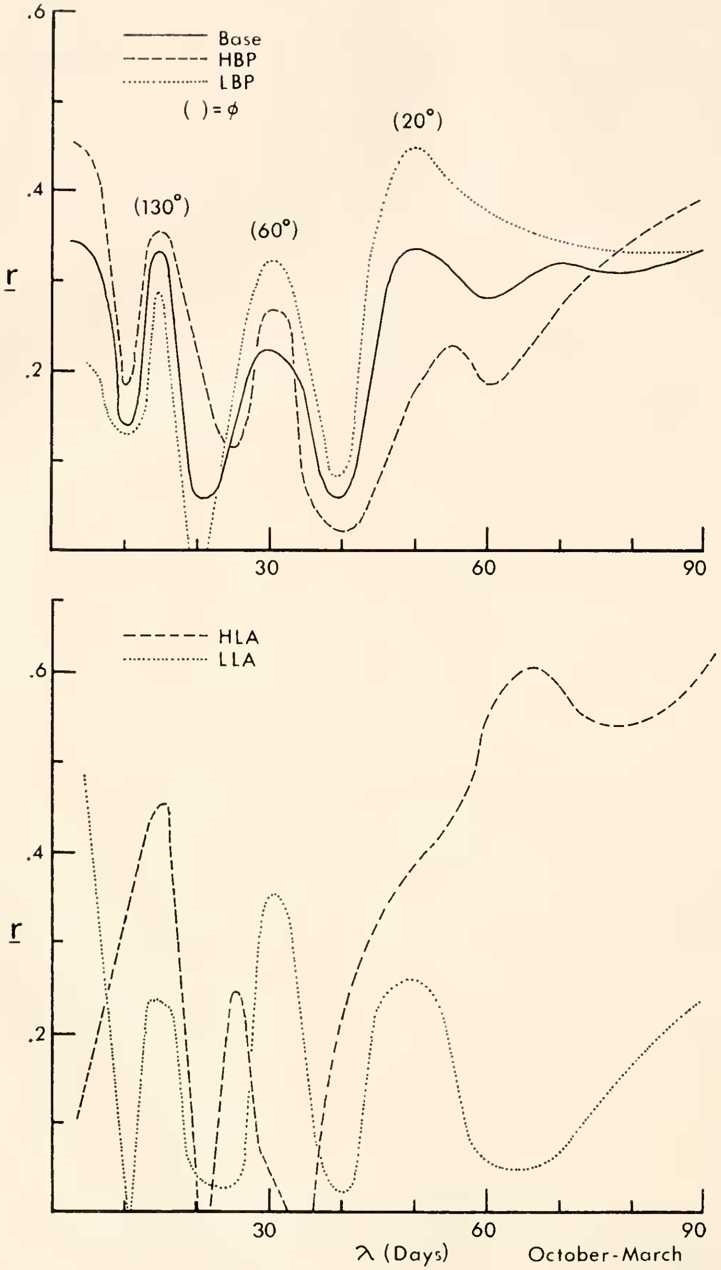
Parameters for significant monthly and annual locomotor activity cycles.

Time segment	Period (days)	ω	ϕ (Degrees)	ϕ (Day)	M	A	Related variables (see text)
April–June	15	24	210	9	−0.73	−1.98	LLA
	20	18	270	15	−1.00	−2.10	HBP, HLA
	30	12	30	3	−0.30	3.35	LBP, LLA
	50	7.3	100	14	−0.95	2.78	HBP, HLA
July–October	5	72	310	4	1.64	−3.30	LBP, LLA
	15	24	170	7	1.08	2.61	HBP, HLA
	27	13.3	120	9	1.15	2.05	LBP, HLA
	43	8.4	220	26	3.38	−2.09	LLA
	50	7.3	310	43	2.41	−3.49	HLA
October–March	15	24	110	5	1.36	5.69	HBP, HLA
	25	14.4	70	5	0.75	3.61	HLA
	30	12	40	3	2.88	2.80	LBP, LLA
	50	7.3	40	6	0.91	4.73	LBP
	65	5.5	160	29	1.59	7.05	HLA
Annual	30	12	52	4	2.25	2.16	LBP, LLA
	55	6.5	52	8	0.18	1.61	HLA
	92	3.9	307	78	2.86	−2.13	LBP, LLA
	105	3.4	60	17	0.09	2.95	HBP, HLA
	162	2.2	147	66	0.69	2.15	HLA
	360	1	110	110	1.91	1.49	LLA

dominant, whereas on days of LBP the 30, 60, and 90 day cycles were most significant. High LA increased the significance of the 55 and 105 day cycles, while LLA was associated with the presence of 30, 60, and 90 day cycles.

Parameters generated from the nonlinear analysis (Table II) are based upon the maximum values of r derived from the data based upon the relative barometric pressure and locomotor activity. These values show slight shifts in period and phase angle (ϕ) when compared to the base spectra (Figs. 6–9) where ϕ was held constant. It is apparent that values of M (mean of LA_{max} time values) for each of the cycles in the three monthly segments varied from negative to positive values, which indicates that they are probably oscillating on a larger period of about a year. This reflects the greater degree of LA_{max} activity prior to 0000 hr (negative values) in April to June and the gradual shift of activity after 0000 hr (positive values) in July to October with an apparent reversal during October to March.

FIGURE 7. Upper graph shows a period analysis of time of LA_{max} during July to October indicating by the solid line revealing several significant cycles at 5, 15, 27, and 43 days and larger periods (> 90 days). On days of low barometric pressure (LBP) the 5, 27, and 50 day cycles are dominant, whereas on days of high barometric pressure (HBP), the 15 day cycle was the most significant. Lower graph shows a similar analysis on days of low activity (LLA) revealing that the 5 day and larger periods were significant, while high activity (HLA) was associated with 15, 27, and 50 day cycles.


Base
HBP
LBP
( ) = φ
(130°)
(60°)
(20°)
.6
.4
.2
r
30
60
90
HLA
LLA
.6
.4
.2
r
30
60
90
λ (Days)
October-March

The dispersion of activity throughout the day during this latter period is also reflected in the larger amplitude values.

The cycles that are dominant in the annual analysis are the result of two major fundamental frequencies which possess specific harmonic characteristics. The first set of cycles (alpha series) oscillates on M which ranges between 0.09–0.69 and is represented by the 55, 105, 162 day cycles. The phase angles indicate that the 55 and 105 day cycles are reinforced about every 105 days, and the 162 day cycle is reinforced by the 55 and 105 day cycles about every 323 days. Thus, the shortest fundamental period would be about 323 days in which the harmonics are 6ω, 3ω, and 2ω with the first synchronous date on November 15, 1976.

The second fundamental series of cycles (beta series) oscillates on M which ranges between 1.91–2.86. The fundamental period may be the 30 day cycle with multiples at $\omega/2$, $\omega/3$, and $\omega/12$ (Fig. 9), and the values of ϕ indicate that the two significant 30 and 90 day cycles are synchronized at days 32–34, 124, 214–216, and 304–308.

By χ^2 analysis the variables of barometric pressure and relative daily activity, which alter the significance of these cycles throughout the year, have a significant ($P = 0.01$) nonrandom association in which high barometric pressure is associated with increased daily activity and low pressure with low daily activity. These associative features are also dichotomous, since high barometric pressure and high activity is significantly associated ($P < 0.01$) with the alpha cyclic series, while low barometric pressure and low activity is associated with the beta cyclic series.

DISCUSSION

The spontaneous group activity over the course of a year in adult male frogs under the conditions of this study show a correlation with the light-dark cycle, ambient barometric pressure and an exogenous lunar perturbation which appears to influence the time and relative level of locomotor activity. Each of these variable factors is present throughout the year, but the change in the L:D ratio appears to be the dominant influence. Absence of a significant diurnal activity pattern with a L:D ratio of < 1.0 indicates that the length of time of the nocturnal period may be the primary influence of increased nocturnal activity, since the reversed pattern was not apparent during the winter. With a decreasing dark period, activity shifts from primarily nocturnal to auroral at the time of the longest days in June. Bush (1963) noted that specimens of *Bufo fowleri* were inactive with a 4L:20D cycle but became quite active as the L:D ratio was altered to 12L:12D and 20L:4D. Further, toads consumed more with the longer photoperiods. Physiological responsiveness of amphibians to photoperiod is variable, since the spawning period of

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FIGURE 8. Upper graph shows a period analysis of times of LA_{max} from October to March revealing basic cycles (solid line) at 5, 15, 30, and 50 days. Only the 5 day cycle was altered significantly under conditions of high barometric pressure (HBP), while the 50 day cycle was more dominant under low barometric pressure (LBP). Lower graph shows analysis of dominant cycles in periods of high locomotor activity (HLA) showing significant enhancement of the 15 and 65 day cycles, whereas a 30 day cycle was most significant with days of low locomotor activity (LLA).

Rana temporaria appears to be controlled by temperature and not by light (van Oordt and van Oordt, 1955); but the spermatogenic cycle in the salamander *Plethodon cinereus* is primarily regulated by photoperiod (Werner, 1969). The

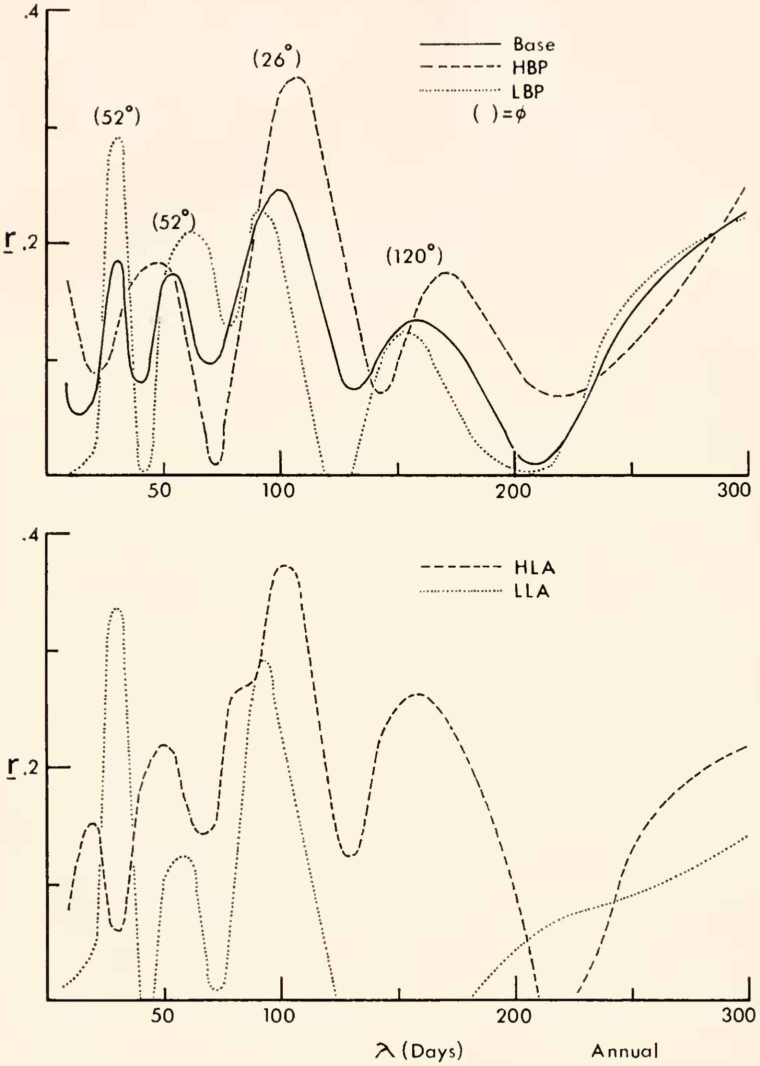

FIGURE 9. Upper graph shows a period analysis of all LA_{max} data collected throughout the year revealing major cycles at 30, 55, 105, and 160 days. Under conditions of lower than the yearly mean barometric pressure (LBP), significant cycles were apparent at 30, 60, 90, and 155 days, whereas with higher than the yearly mean barometric pressure (HBP) the dominant cycles were at 50, 105, and 175 days. Lower graph shows a similar analysis of those cycles most dominant when frogs were least active (LLA), present at 30, 60, and 90 days, while cycles apparent on days of high activity (HLA) were at 50, 105, and 165 days.

increase in nocturnal activity with the beginning of longer days may be an independent but synchonized part of the initiation of sexual activity after the spring equinox.

The influence of temperature on amphibian behavioral activity has been noted by several authors. Early observations by Torelle (1903) of specimens of *Rana virescens virescens* (*Rana pipiens pipiens*) found that at temperatures < 10° C, frogs moved away from light; whereas with temperatures > 30° C frogs were attracted to light. Higginbotham (1939) observed that with a 10° C rise in temperature there was a doubling to tripling of the amount of activity of toads (*Bufo americanus* and *Bufo fowleri*) but no alteration in the overall pattern of activity. *Bufo fowleri* also appears to be most active between 19.6–24.7° C in April to May (Martof, 1962) and consumes twice as much food with a 10° temperature rise from 21° C to 31° C (Bush, 1963). These responses in behavioral activity with change in ambient temperature is of importance in the interpretation of the present data. Over the course of a year the temperature range was held at 8° C and was within the limits observed by Martof (1962) where toads are most active. However, within the temperature range of the present study there was a nonsignificant, and somewhat negative relationship, between total daily activity and the prevailing temperature. Within any monthly period, the maximal temperature range was only 3° C, which was considerably less than the 10° C differential associated with variations in total activity of toads (Higginbotham, 1939; Bush, 1963). Thus, while it is possible that the slight variations in temperature could modify portions of the observed activity, clear relationships were not as apparent as those associated with the light-dark cycle, lunar frequencies and barometric pressure.

The exogenous lunar influence which has been described by others (Ralph, 1957; FitzGerald and Bider, 1974) to affect amphibian activity patterns was also apparent in the present study. Time of high transit was associated with an enhancing and depressing effect during daylight hours in winter and spring to early summer, respectively, and represents a possible mechanism to induce oscillatory patterns in activity as seen in the activity isogram for April to June. The patterns may be modified with the varying length of daylight hours. The fact that the activity isogram in the fall is not a replica of the pattern observed in the spring may reflect the reversal in activity levels of these animals during daylight to this exogenous influence.

The cyclic patterns of locomotor activity, as revealed by wave analysis, in the three major monthly periods and for the entire year appears to represent a consistent phenomenon. That is, the time of maximal activity each day occurred at a similar time period at a later date which was determined by the dominant cycle present. This is best exemplified by the lunar cycle which approximates 30 days (beta-series) and is apparent in the isogram of locomotor activity for April to June. But in addition to this more obvious lunar cycle, there are other cycles that are generated by lunar and solar movement which can give rise to tidal harmonics (Godin, 1972). The principal lunar constituent (M_2) has a period of 12.42 hours and other cycles "beat" against M_2 giving rise to a lunar fortnightly tide (13.66 days), a monthly modulation of 27.57 days and a solar semiannual tide of 182.9 days. Thirty-four separate tidal frequencies have been identified (Godin, 1972), each of which can generate their own harmonics which can reinforce or dampen one another. The activity cycles which are observed in frogs may be affected by such tidal harmonics, the relative dominance of each reflected in the value of r at the optimal phase angle. For example, the characteristics of the alpha-series of cycles, with a fundamental period of 323 days, suggests that it may be related to the eclipse year of 346.6 days (a "beat" frequency of the synodic and draconitic months), since the first synchronous date of all harmonics was nine days after a lunar eclipse on 11/6–7/76. The next ideal synchronous date is 10/5/77 which is between two eclipses on 9/27/77 (lunar) and 10/12/77 (solar) (*American Ephemeris and Nautical Almanac*, 1976, 1977). Confirmation of such a relationship would require analysis of locomotor activity over a period of several eclipse years.

Of interest is that these cycles are present from October to March, a period characterized by continuous 24 hour activity in the absence of a defined diurnal pattern. Period analysis shows that they are quite similar to the cycles observed in April through June and thus appear to be independent of the light-dark cycle and may be similar to the lunar perturbations observed by Ralph (1957) in the salamander kept in continuous darkness. These oscillations may be expressed as overt activity patterns when the light-dark effect is significant to induce a defined nocturnal pattern. While it may be argued that these are statistical cycles and not overt, patterns can be identified in composite isograms. Further, they can be mathematically described as a first order approximation to a sine wave which allows a possible means of prediction (Table II).

An additional feature of the wave analysis is that other variables such as barometric pressure and relative levels of locomotor activity can be related to two fundamental cycles which might otherwise be difficult to detect. Brown *et al.,* (1955) noted that oxygen consumption in the salamander *Triturus* was inversely related to barometric pressure but not sharply defined. Even in the present study a significant inverse relationship can be derived between absolute daily lomomotor activity and absolute barometric pressure during May ($r = -0.0418$: $P = 0.02$), but it is not consistent at other months. The present analysis reduces the apparent "biological noise" by defining specific days and times of LA_{max} which can be correlated with prevailing barometric pressure.

The sensitivity of animals to atmospheric pressure changes has been observed in aquatic invertebrates such as *Carcinus* (Naylor and Atkinson, 1972) and to hydrostatic pressure as in the amphibian tadpoles, *Rana silvatica* and *Amblystoma* (Johnson and Flagler, 1951), which in both cases increase their activity with an increase in pressure. Conversely, in a terrestrial form such as a newly hatched chick, increased activity is associated with a decrease in barometric pressure (Bateson, 1974).

In the present study this apparent correlation of barometric pressure to activity level was specific for certain cycles. The relationship of these two variables is unclear, but lunar and solar movement also generates atmospheric tides in addition to marine tides (Siebert, 1961). The lunar component can induce a barometric variation of 0.001 millibars (0.025 mm Hg), while the solar component can induce a variation up to 0.03 millibars (0.76 mm Hg). Detection of such variations in barometric pressure exclusive of the daily weather perturbations might induce the observed cyclic patterns. In view of the dichotomy of the alpha and beta series of cycles which are associated with specific variables, the resultant patterns of activity may reflect two independent receptor-effector behavioral systems.

In summary, the daily and annual solar light pattern appears to be related to the diurnal and nocturnal activity of the adult male frog, *Rana pipiens,* but it has activity patterns of specific periodicities which are similar to lunar periodicities. Additionally, there are significant correlations between these cyclic patterns with the prevailing barometric pressure and the level of locomotor activity. The general behavior patterns of this amphibian over the course of a year exhibits several correlates to basic geophysical forces. The significance of these relationships may be reflected in similar activity patterns of organisms, such as insects (see reviews of Harker, 1958; Corbet, 1960), which enter into the amphibian food chain. Such synchrony could increase the probability of successful feeding and ultimate survival. Variations in intestinal transport activity (Robertson, 1976) may reflect the general physiological cycling coherent with these food gathering processes.

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SUMMARY

The spontaneous locomotor activity (LA) of adult male frogs (*Rana pipiens,* Northern variety) was monitored throughout the year in an apparatus which detected vertical water movements. Frogs exposed to the seasonal change in ambient light and maintained at a constant mean annual temperature of 19.3 ± 3.1° C exhibited significant correlations of activity to the light-dark cycle, barometric pressure and lunar perturbations. When the light : dark ratio was < 1.0 (October to March) frogs displayed "random" activity throughout the 24 hr period; but with the L : D ratio between 1.0–1.45 activity was primarily nocturnal between 2100–0000 hr and at sunrise, while with a L : D Ratio > 1.45 maximal activity occurred at sunrise. Activity also was correlated with time of lunar high transit (HT) where occurrence of HT during daylight hours in April to June was associated with depressed activity, while HT during daylight in October to March was coincident with elevated period of activity. Use of a FORTRAN IV Program to analyze time of maximal LA each day throughout the year revealed oscillatory behavior patterns with periods similar to lunar tidal cycles. An alpha-series of cycles (55, 105, and 162 day periods) were significantly associated and dominant on days of high barometric pressure (above the annual mean of 761 mm Hg) and characterized by high levels of activity (above the annual mean of 313 events/day). A beta-series (30, 60, 90 day cycles) was dominant on days of low barometric pres-

sure (< 761 mm Hg) and coincident with low levels of activity (< 313 events/day). Spontaneous activity of frogs apparently is not random, but reflects an association with basic geophysical forces which elicit a complex but definable behavior pattern.

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